Effect of Bilateral Destruction of the Subretrofacial Area on Receptor Mechanisms of Neuroprotective Effect of GABAergic Agents

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 125, No. 2, pp. 162-164, February, 1998. Original article submitted April 24, 1997.

Both GBA_A (muscimol, gaboxadol, and isonipecotate) and $GABA_B$ (baclofen) receptor agonists produce marked neuroprotective effect during total brain ischemia. The antagonists of $GABA_A$ receptors bicuculline and picrotoxin attenuate the effect of muscimol, and the $GABA_B$ receptor antagonists hydroxysaclofen and aminovaleriate decrease the effect of baclofen. The GABAergic substances protect the brain via GABA receptors of both types. The effect of the GABA agonists is central in nature.

Key Words: *GABA receptors; neuroprotectors; brain ischemia*

γ-Aminobutyric acid (GABA) is the main inhibiting transmitter in the brain. Its effect is realized via different signal-transducing systems: 1) ionotropic $GABA_A$ receptors, which are Cl^- channels that hyperpolarize cells due to inflow of chloride ions; and 2) metabotropic $GABA_B$ receptors, which open K^+ and close Ca^{2+} channels via $G_{i/o}$ proteins and inhibit adenylyl cyclase [5,6,13]. Recently, $GABA_B$ receptors were cloned [8]. The agonist of $GABA_B$ receptors baclofen has a pronounced neuroprotective effect (NPE) during brain ischemia in the rodents [2,3,7,9]. The data on muscimol, an agonist of $GABA_A$ receptors, are equivocal [7,10,12]. The effects of GABA receptor antagonists were studied only for NPE of baclofen [7]. Our aim was to elucidate the receptor mechanisms of NPE of GABA-ergic agents using two independent methods: 1) comparison of activity of selective agonists of different GABA receptors and 2) blockade of the effects by selective antagonists [5,6,13].

MATERIALS AND METHODS

The study was carried out on 372 CBA and C57B1 mice of both sexes weighing 18-25 g. The following substances were used: baclofen (Sigma, RBI), muscimol and bicuculline (Serva), gaboxadol×HCl (THIP), isonipecotic acid, 5-aminovaleric acid×HCl, and 2-hydroxysaclofen (RBI). Picrotoxin was provided by Dr. K. S. Raevskii. The substances were dissolved in water (bicuculline was dissolved with slight acidification). They were injected subcutaneously (10 ml/kg) or into the left lateral cerebral ventriculum (3 μl; volume of injected hydroxysaclofen was 6 μl). The optimal conditions for manifestation of pharmacological effect were chosen on the basis of the dose-response and time-response curves. There were no differences in the control mice during a 15-20-min interval. The decapitation model of total brain ischemia was used together with estimation of gasping duration. The series were compared according to nonparametric Mann-Whitney's *U* test [4].

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RESULTS

Both baclofen, a $GABA_B$-agonist, and three different $GABA_A$ agonists (gaboxadol, muscimol, and isonipecotate) exert strong NPE (Table 1). Muscimol and isonipecotate are particularly efficient after intracerebroventricular injection as compared with subcutaneous injection: an equal effective dose of mus-

0007-4888/98/0002-0139$20.00

TABLE 1. Effect of $GABA_A$ and $GABA_B$ Receptor Agonists on the Resistance to Total Brain Ischemia

Substances		Injection period, min	Dose, mg/kg [μmol/kg]		Gasping duration, sec	
			s.c.	i.vent.	s.c.	i.vent.
CBA	Control	15-120	—	—	17.4 (15-23) n=20	18.4 (15-23) n=20
	Gaboxadol ($GABA_A$-agonist)	75	40 [284]	—	45.1** (21-40) n=11	—
	Muscimol ($GABA_A$-agonist)	60	5 [43.8]	0.015 [0.132]	40.0** (31-50) n=5	45.0** (35-56) n=12
Ourbred	Control	15-90	—	—	15.9 (14-18) n=8	16.7 (19-19) n=9
	Isonipecotate ($GABA_A$-agonist)	30	1000 [7752]	1/5 [12]	19.3* (17-21) n=6	27.0** (25-34) n=10
C57B1						
	Control	60-120	—	—	18.9 (15-24) n=16	—
	Baclofen ($GABA_B$-agonist)	120	30 [140]	—	46.4** (32-58) n=6	—

Note. The range of variation is given in parentheses; n is the number of experiment; i.c. — intracutaneous injection; i.ventr. — intraventricular injection. *p<0.01 and **p<0.001 in comparison with control.

cimol in the first case is 300-fold smaller than that of isonipecotate, which poorly penetrates into the brain and provides a weak protection by subcutaneous injection even in a 700-fold dose. This testifies to the central mechanism of the effect of $GABA_A$ agonists.

The $GABA_A$ antagonists bicuculline and picrotoxin produce protective effect, but NPE of muscimol injected either subcutaneously or intraventricularly against the background of these substances is low (Table 2). Therefore, bicuculline and picrotoxin demonstrate dual agonist-antagonist properties. Blockade of muscimol-induced NPE is most pronounced after 2 injections of bicuculline or after administration of bicuculline in combination with picrotoxin (in comparison with control, NPE is decreased in both cases by 62% and, respectively, by 67 and 92% in comparison with the antagonists).

After intracerebroventricular injection, the $GABA_B$ antagonists do not affect brain resistance to ischemia, but markedly decrease the NPE of baclofen (Table 3): aminovaleriate by 63%, and the selective blocker hydroxysaclofen by 75% (the blockade was much weaker when hydroxysaclofen was injected 45-60 min prior to baclofen).

Thus, both $GABA_A$ and $GABA_B$ receptor agonists produce pronounced NPE. The effect of muscimol is drastically attenuated by $GABA_A$ antagonists bicuculline and picrotoxin, while that of baclofen by $GABA_B$ antagonists hydroxysaclofen and aminovaleriate. The coincidence of results obtained by twoby two independent methods (comparison of effects of selective receptor agonists and blockade with selective antagonists) indicates that NPE is mediated via GABA receptors of both types. It differs strikingly from NPE of catecholamines and their derivatives, which is mediated only via α_2-adrenoceptors [1]. However, the most important GABA effect, namely, the decrease in functional cerebral activity, is also realized via GABA receptors of both types [5,6,11]. It should be noted that in addition to NPE, the agonists of

TABLE 2. Effect of $GABA_A$ Receptor Antagonists on the Neuroprotective Action of Muscimol in Mice CBA

Antagonists, dose mg/kg [μmol/kg]	Agonists			
	Subcutaneous injection		Intraventricular injection	
	Agonist-free	Muscimol, 5 mg/kg [43.8]	Agonist-free	Muscimol, 0.015 [1.132]
Antagonist-free	17.4 (15-22) n=20	40.0[b] (31-50) n=8	18.4 (15-23) n=20	45.0[b] (35-56) n=12
Bicuculline, 0.7 [1.9]	22.8[b] (20-27) n=5	—	—	38.7[b,d,f] (33-44) n=6
Bicuculline[1], 0.7 [1.9]	19.7[a] (16-23) n=10	25.7[b,d,g] (20-33) n=6	—	28.4[b,e,h] (25-30) n=5
Picrotoxin, 1.5 [2.5]	27.8[b] (23+4) n=5	—	—	35.6[b,c,g] (29-43) n=7
Picrotoxin + bicuculline, 1.5 [2.5]	27.0[b] (21-32) n=5	—	—	28.5[b,h] (25-33) n=6

Note. All antagonists were injected 15 min prior to muscimol (bicuculline was repeatedly injected 30 min after muscimol). Comparisons here and in Table 3: with the control group [a]p<0.05, [b]p<0.001; with antagonist [c]p<0.1, [d]p<0.01, [e]p<0.00; with agonist: [f]p<0.1, [g]p<0.01, [h]p<0.001.

TABLE 3. Effect of $GABA_B$ Receptor Antagonists on the Neuroprotective Action of Baclofen in Mice C57Bl

Antagonists	Substances		Agonist-free	Baclofen	
	Dose mg/kg [μmol/kg]	Time (min)		1 h	2 h
Antagonist-free		15-60	18.9 (15-24) *n*=16	37.3[b] (33-44) *n*=6	46.4[b] (32-58) *n*=14
Aminovalerianic acid	9 [76]	15	19.8 (16-24) *n*=6	29.8[b,e,i] (23-38) *n*=16	29.0[b,d,h] (20-38) *n*=1
Hydroxysaclofen	0.5 [1.9]	15	17.9 (14-21) *n*=10	—	30.1[b,e,h] (23-39) *n*=10
	0.5 [1.9]	30	17.9 (14-21) *n*=10	—	22.6[d,h,i] (15-30) *n*=11
	1 [3.8]	15	18.3 (16-23) *n*=4	—	24.0[d,g,i] (18-30) *n*=5
	1 [3.8]	30	18.3 (16-23) *n*=4	—	26.0[b,d,h] (20-34) *n*=6

Note. [b]$p<0.05$ compared with agonist; [i]$p<0.01$

$GABA_B$ [2] and $GABA_A$ receptors produce pronounced hypothermia. After intracerebroventricular injection, muscimol and, to a greater extent, isonipecotate are much more active (by 2-3 orders of magnitude), while hydroxysaclofen and aminovaleriate block NPE of subcutaneous baclofen. This indicates that NPE of GABAergic substances is central in nature.

This work was supported by the Russian Foundation for Basic Research, project No. 97-04-49139.

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